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Cardioprotective Effect of a Medicinal Mushroom, *Ganoderma lucidum* Against Adriamycin Induced Toxicity

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Abstract: The present study was conducted to evaluate the cardioprotective role of a medicinal mushroom, *Ganoderma lucidum* against the toxicity of an anticancer drug adriamycin. Wistar rats were administered with adriamycin (1.5 mg kg^{-1} b.wt.) for three weeks to induce cardiotoxicity. Another group was treated with adriamycin and *Ganoderma* extract (250 mg kg^{-1}) for 30 days. Adriamycin treatment resulted in increased serum levels of marker enzymes such as alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK). Besides increasing the lipid peroxidation (LPO), adriamycin significantly reduced antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) in the heart tissue. Level of reduced glutathione (GSH) was also significantly reduced. However, in *G. lucidum* extract treated rats the alterations were not significant, but found to be nearer to the control. The study shows that *G. lucidum* extract exhibits significant antioxidant property and protect the heart from free radical mediated toxicity of adriamycin.

Key words: Adriamycin, mushroom, antioxidant, cardioprotection

INTRODUCTION

Adriamycin, also known as doxorubicin, is an anthracyclin antibiotic used to treat a variety of solid tumors and hematopoietic malignancies in children and adults (O'Bryan *et al*., 1973; Tan *et al*., 1973) and is popular for its promising anticancer potential. However, its fatal cardiotoxicity subdued enthusiasm for this drug. Patients receiving this drug and animal experimentation reveal its life threatening cardiotoxicity (Mason, 1979; Rowan and Chlebowski, 1979; Lenaz and Page, 1976; Henderson and Frei, 1980). Adriamycin cardiotoxicity is mediated by multiple mechanisms, primarily by free radical generation, membrane lipid peroxidation, mitochondrial to macromolecule damage (Kalyanaraman *et al*., 1980; Jackson *et al*., 1984; Doroshow, 1991; Xu *et al*., 2001) leading to irreversible myocardial damage with fatal congestive heart failure (Von Hoff *et al*., 1979). Adriamycin has been shown to cause deficiency of antioxidants in different animals experimentation (Revis and Marusic, 1978; Doroshow *et al*., 1980). Low level of antioxidant enzymes such as superoxide dismutase and catalase in cardiac muscles make it more susceptible to adriamycin (Doroshow *et al*., 1980; Naidu *et al*., 2002). Increased generation of reactive oxygen species such as O_2, OH and H_2O_2 (Doroshow, 1983; Doroshow and Davies, 1986; Lee *et al*., 1991; Wong *et al*., 2004), high myocardial lipid peroxidation and decrease in antioxidant enzymes (Naidu *et al*., 2002) in adriamycin and doxorubicin treated animals show the vulnerability of heart muscles.

As evident from the vast reports, it is clear that adriamycin toxicity is because of free radicals. And hence, identification of any agent that would minimize the reactive radical generation and increase antioxidant defense would be a great hope to patients. Administration of adriamycin with another agent that would block its free radical generation without interfering the anticancer effect has been a long existing goal. In the line, use of several cardioprotective agents both synthetic and herbal derived products have been reported to offer protection against adriamycin and doxorubicin cardiotoxicity. Probucol, a lipid lowering drug (Siveski-Iliskovic *et al*., 1994; Li *et al*., 2000), cranberry (Elberry *et al*., 2010), carvedilol (Ibrahim *et al*., 2010), *Schisandra chinensis*, a Chinese herbal medicine (You *et al*., 2006), carnosine (Ozdogan *et al*., 2011), *Ephedra nebrodensis* (Shah *et al*., 2009), amifostine (Bolaman *et al*., 2005), spirulina (Khan *et al*., 2005), *Lagenaria siceraria* (Fard *et al*., 2008), metformin, a hypoglycemic drug (Aleisa *et al*., 2010) have been shown to protect heart from adriamycin and doxorubicin toxicity.

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Role of herbs and their products in treating cardiovascular problems has been extensively reviewed (Burta *et al*., 2008; Upaganlawar *et al*., 2011). Antioxidant mediated amelioration of experimental cardiomyopathy by garlic (Vibha *et al*., 2011), garlic with propranolol (Asdaq *et al*., 2008), *Bacopa monnieri* (Nandave *et al*., 2007), *Tribulus terrestris* (Ojha *et al*., 2008), fruit juice of *Lagenaria siceraria* (Upaganlawar and Balaraman, 2010), grape seed extract (Saalu *et al*., 2009), *Ficus hispida* (Shanmugarajan *et al*., 2008) is an evident for the potency of plants in treating heart diseases.

Though there are studies on plants and other products on cardioprotection, reports on mushroom role are scanty. Mushrooms are popular for their nutritional and medicinal values but have received little attention in cardioprotection despite rich antioxidant content. In the present study, *Ganoderma lucidum* was used for its cardioprotection against adriamycin induced toxicity. *G. lucidum* has been reported to have immunomodulating, antiatherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radioprotective, sleep promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, antifibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, anti-aging and anti-ulcer properties (Wasser and Weis, 1997; Chang and Buswell, 1999; Jong and Birmingham, 1992; Gao *et al*., 2002; Wasser and Weis, 1999; Wasser, 2002). *G. lucidum* is also known for its antioxidant activity and effective scavengers of oxidative radicals (Joseph *et al*., 2009; Mau *et al*., 2002). However, studies on the antioxidant mediated cardioprotective role of *G. lucidum* are scanty. And hence, the present study was conducted.

MATERIALS AND METHODS

Mushroom specimen: *G. lucidum* specimens were collected from wild and identified. Fresh specimens were cut into small pieces, dried and powdered. The powdered sample was ethanol extracted using Soxhlet apparatus. The extract (GDE) was concentrated in rotary vacuum evaporator and stored at 4°C for further use.

Chemicals: Adriamycin (ADR), as doxorubicin hydrochloride was purchased from Fresenius Kabi Oncology Ltd., Solan, India. Biosystems Diognostics kits (BioSystems S.A. Barcelona, Spain) were used for AST, ALT, LDH and CK estimation. All other chemicals used were analytical grade procured from Himedia Laboratories Pvt Ltd., Mumbai, India and Sigma Alridge, UK.

Experimental protocol: Wistar rats weighing 250±10 g b.wt. were used for the experiments. They were housed in SASTRA Central Animal Facility and maintained in conditions approved by the ethical committee. The study protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA:94/SASTRA/IAEC/RPP). The rats were provided with standard pelletized diet and water *ad libitum*. Rats were divided into four groups with 6 animals each. Group I was control (CON), administered with saline intraperitoneally (i.p.); Group II was administered with Adriamycin (i.p.) 1.5 mg kg^{-1} b.wt. daily for three weeks; Group III was receiving only *G. lucidum* ethanol extract (GDE, 250 mg kg^{-1} b.wt.) orally and the Group IV was administered with ADR (i.p.) and GDE orally. GDE administration was continued for 30 days in groups II and III.

At the end of the experiments rats were anesthetized by CO_2 asphyxiation and blood samples were collected from retro orbital vein. Serum was separated for biochemical assays. Heart was excised quickly and washed with physiological saline. Tissue homogenate was prepared in 0.1 M tris-HCl buffer (pH 7.4) and used for the determination of LPO, GSH, GPx, GST, CAT and SOD.

Serum biochemical assays: Serum was used for the estimation of cardio marker enzymes such as ALT, AST, LDH and CK using Biosystem diagnostic kits (Barcelona, Spain).

Assay of lipid peroxidation: Heart tissue homogenate was used for the estimation of lipid peroxidation following the method described by Ohkawa *et al*. (1979) in which malondialdehyde (MDA) released was used as the index for lipid peroxidation.

Reduced glutathione (GSH): GSH was estimated by the method of Ellman (1959).

Glutathione peroxidase (GPx): GPx estimation was done following the method of Paglia and Valentine (1967).

Glutathione-S-transferase (GST): Assay of GST was done by the method of Habig *et al*. (1974).

Catalase (CAT): CAT activity was measured following the method described by Takahara *et al*. (1960).

Superoxide dismutase (SOD): SOD level in the heart homogenate was measured by following the method of Misra and Fridovich (1972).

Statistical analysis: Statistical analysis of the results was done by one way analysis of variance (ANOVA) using GraphPad Prism 5 software, followed by Dunnett's comparison test for significance. Significance was set at ($p<0.05$) and($p<0.01$). Results are presented as Mean±SE.

RESULTS

Drastic alterations in the level of serum marker enzymes AST, ALT, LDH, CK and lipid peroxidation product MDA and antioxidant enzymes were noted in ADR treated rats. The activity of AST and ALT was significantly ($p<0.05$) higher in ADR treated rats when compared to control rats. However, in GDE+ADR treated groups the levels were nearer to that of control values. Similarly, significant ($p<0.05$) increase in LDH and CK activity was noted in ADR treated groups when compared to the control groups. The enzyme levels in GDE+ADR administrated rats did not show significant alterations from that of control group. In group III rats treated with GDE alone no significant changes in the serum marker enzyme level were noted with reference to control. Significantly increased LPO ($p<0.05$) was also evident in the heart tissue of rats administered with ADR when compared to control group. MDA level in GDE+ADR treated groups showed no significant difference from that of control value, an evidence for reduced lipid peroxidation (Table 1).

Antioxidant enzymes GPx and GST in heart tissue were significantly ($p<0.05$) reduced in ADR treated groups, associated with significant reduction of GSH. The levels were nearer to the control values in ADR+GDE treated groups. Similar results, with significant ($p<0.05$) reduction of antiperoxidative enzymes SOD and CAT were also noted in ADR administered groups. However, in rats treated with GDE and GDE+ADR, the enzyme activities were close to control group (Table 2).

DISCUSSION

As observed in the present study, ADR treatment has caused significant alterations in the levels of serum marker enzymes such as AST, ALT, LDH and CK, indicating a severe cardiotoxicity exhibited by ADR. Significant elevation of these cardiac marker enzymes level in serum can be attributed to leakage from mitochondria due to adriamycin induced mitochondrial damage (Shakir and Rasul, 2009). In addition to elevated marker enzymes, considerable alterations of antioxidant enzymes in cardiac tissues show the grave cellular damages caused by the ADR. Though there are several reasons such as inhibition of nucleic acid and protein synthesis (Buja *et al*., 1973; Arena *et al*., 1974; Monti *et al*., 1995), mitochondrial (Gosalvez *et al*., 1979) and lysosomal damages (Singal *et al*., 1985) ascribed for the anthrocycline mediated cardiotoxicity, major factors responsible for the toxicity include free radical formation (Singal *et al*., 1987; Kalyanaraman *et al*., 1980; Doroshow, 1983) and lipid peroxidation (Singal *et al*., 1985; Singal *et al*., 1987; Myers *et al.,* 1977).

Adriamycin has been shown to cause membrane damage and bioreductive activation leading to formation of free radicals such as hydrogen peroxide (H_2O_2), superoxide anions and hydroxyl radicals (Lown *et al*., 1982; Keizer *et al*., 1990). Increased free radicals and reduced endogenous antioxidants, with subsequent increase in oxidative stress lead to slow loss of myofibrils and vacuolization of myocardial cells (Christiansen and Autschbach, 2006). These cellular damages might have resulted in the leach out of these enzymes to blood stream resulting in elevation as observed in the ADR treated groups. Higher lipid peroxidation as evident from increased MDA level in ADR treated rats was another evidence for the cardio toxicity.

Table 1: Cardio marker enzyme levels in the serum and LPO in the heart of rats in different treatment groups

Experimental groups	AST	ALT	LDH	CK	LPO
	----------(U L^{-1})----------				(nmol mg^{-1} protein)
Control	109.52±2.52	131.51±2.76	192.0±1.36	93.17±1.17	34.83±1.13
ADR	233.34±1.40**	217.72±1.80**	286.15±2.06**	182.51±1.97**	99.12±2.12**
GDE	99.67±1.88*	128.81±2.88	208.75±2.32*	98.83±1.19	35.50±1.99
ADR+GDE	112.82±1.95	138.12±1.48	209.85±1.09*	97.52±2.20	42.17±1.94*

ADR: Adriamycin, GDE: Ganoderma extract, Values are Mean±SE (n = 6), Significance at **$p<0.05$ and *$p<0.01$: Control vs. ADR, GDE, ADR+GDE

Table 2: GSH and antioxidant enzyme status in the heart tissues of different experimental groups

Experimental groups	GSH	GPx	GST	SOD	CAT
Control	78.83±1.49	33.83±1.62	26.83±1.01	21.50±1.84	16.67±1.66**
ADR	30.33±1.82**	10.50±0.76**	12.21±1.56**	7.67±0.55**	6.67±0.42
GDE	71.33±2.83*	28.17±1.62*	23.33±1.16	18.50±1.76	14.17±.0.79
ADR+GDE	73.83±1.60	31.17±0.70	19.83±3.19	21.23±1.77	15.17±1.07

Values are Mean±SE (number = 6), Significance at **$p<0.05$ and * $p<0.01$*: Control vs. ADR, GDE and ADR+GDE. Values are expressed as GSH (nmol g^{-1} tissue), GPx (umol GSH oxidized/min/mg protein), GST (U/min/mg protein), SO (U/g protein), CAT (umol/min/mg protein)

The myocardium has been shown to be more susceptible to free radical damages than any other tissues because of comparatively low level of antiperoxidative enzymes, catalase and superoxide dismutase and also increased suppression of glutathione peroxidase by adriamycin. Shakir and Rasul (2009) studies have also shown increased myocardial lipid peroxidation and decrease in antioxidant enzymes in doxorubicin treated mice (Myers *et al*., 1977; Naidu *et al*., 2002). Adriamycin has caused myocardial antioxidant deficit in different animal species (Revis and Marusic, 1978; Doroshow *et al*., 1980). The reduction in the level of GPx, GST and GSH and also of SOD and CAT in the ADR treated rats in the present study is in accordance with these reports.

Because of the severe free radical mediated cardiotoxicity of the anthracycline anticancer dugs, there has been a need to minimize the free radical generation by adjunct therapy with antioxidant compounds (Liu *et al*., 2002). One of the first antioxidants used against adriamycin cardiomyopathy was vitamin E with promising results (Myers *et al*., 1977). Subsequently several antioxidants such as ascorbic acid (Shimpo *et al*., 1991), reduced glutathione (Yoda *et al*., 1986), selenorganic compound PZ-51 (Pritsos *et al*., 1992), oleanolic acid and urosolic acid (Balanehru and Nagarajan, 1992), ambroxol (Nowak *et al*., 1995) were tried with limited success. In the line, Probucol, a lipid lowering agent was found to offer better protection against adriamycin toxicity (Siveski-Iliskovic *et al*., 1995; Li and Singal, 2000). Free radical scavengers such as melatonin and alpha-lipoic acid have been proved to ameliorate myocardial toxicity induced by doxorubicin (Liu *et al*., 2002). In addition, N-acetyl cysteine and β-carotene (Abdel Baky *et al*., 2009), squalene, an isoprenoid (Farvin *et al*., 2009) and Vitamin E (Onyesom *et al*., 2007) were also shown to be cardioprotective. All these studies provide strong evidence for the role of antioxidants in preventing cardiotoxicity.

Eventhough many mushrooms species have been reported to have antioxidant properties (Ramkumar *et al*., 2010), not much studies on the mushroom mediated protection of cardiotoxicity are available. In the present study, in rats treated with GDE marker enzyme levels were not affected by the ADR treatment, an indication for the protection offered by the mushroom against ADR toxicity. *G. lucidum* treatment has also caused considerable elevation of GSH which is the co-factor for antioxidant synthesis and the antioxidant enzymes, GST, GPx, GSH, SOD and CAT indicating antioxidant defense offered by the mushroom. Similarly, reduced MDA content in ADR+GDE treated rats is an evidence for reduced lipid peroxidation. The cardio protective role of *G. lucidum* can be due to its rich antioxidant chemicals such as triterpenes and polysaccharides (Wachtel *et al*., 2004; Liu *et al*., 1997), polysaccharide peptide complex, phenolics (Sun *et al*., 2004; Mau *et al*., 2002; Mau *et al*., 2005). Phenolics, flavones and ascorbic acid contents of *G. lucidum* were shown to exhibit free radical scavenging effects (Rajasekaran and Kalaimgal, 2011). Cherian *et al*. (2009) reported free radical scavenging and increased antioxidant enzymes in *G. lucidum* extract treated mice. Dose dependent increase in antioxidant activity of *G. lucidum* extracts in ethanol induced cardiotoxicity has been reported by Wong *et al*. (2004). *G. lucidum* with its effective anticancer, immunomodulatory, antiinflammatory and antioxidant potential can be an ideal mushroom to reduce free radical mediated cardiotoxicity by adriamycin.

CONCLUSION

In the present study, *G. lucidum* extract has reduced the severity of cardiotoxicity induced by the anticancer drug adriamycin by minimizing free radical generation or scavenging the free radicals and also by increasing the level of glutathione content and the different antioxidant enzymes. By its natural anticancer potential *G. lucidum* may support the adriamycin in preventing cancer progression. However, further study is needed to validate the combiantion effect in curing the cancer.

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